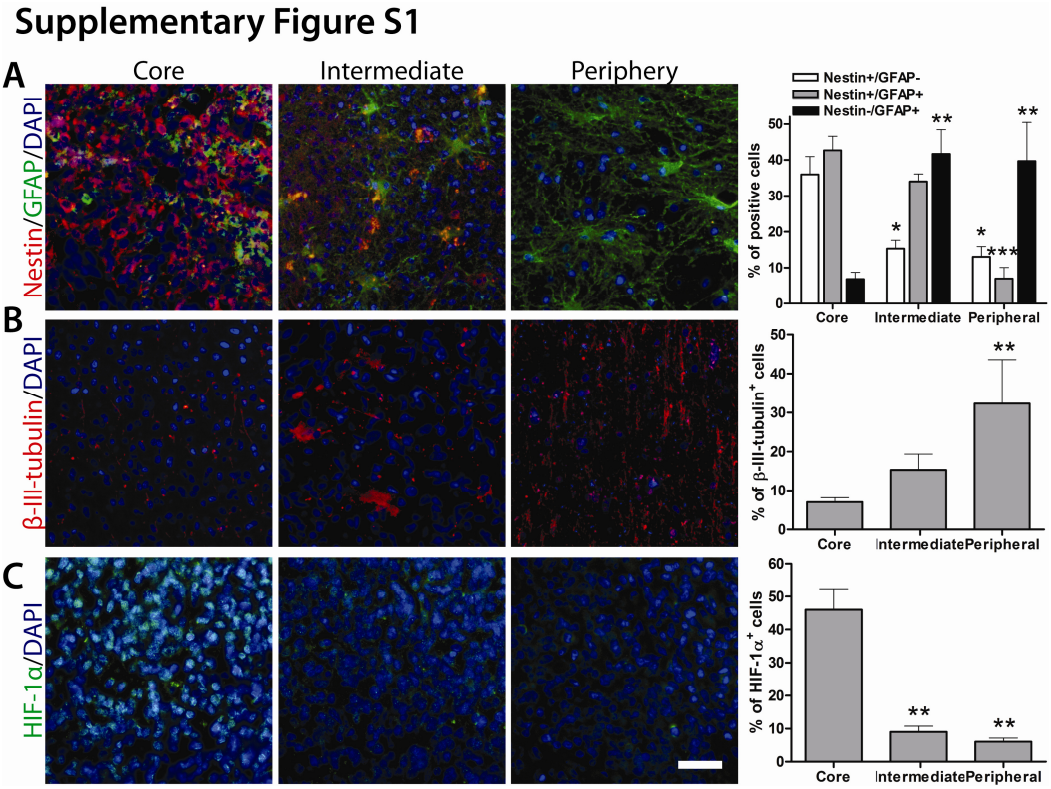
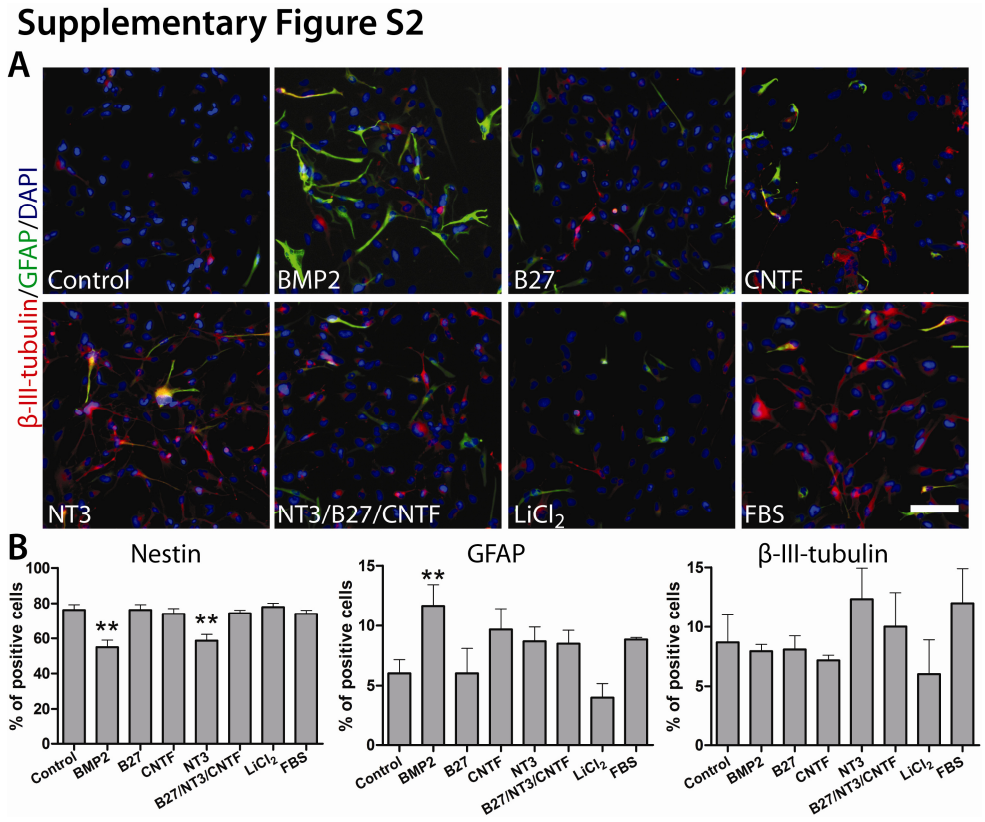
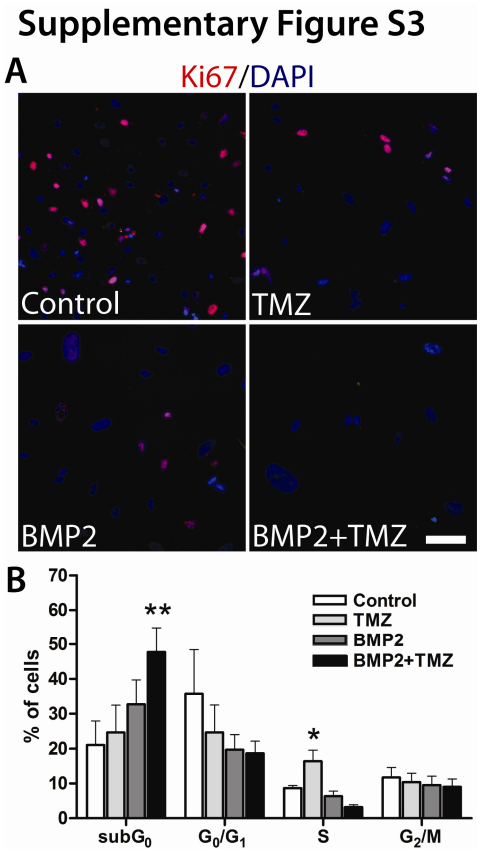
SUPPLEMENTARY FIGURES

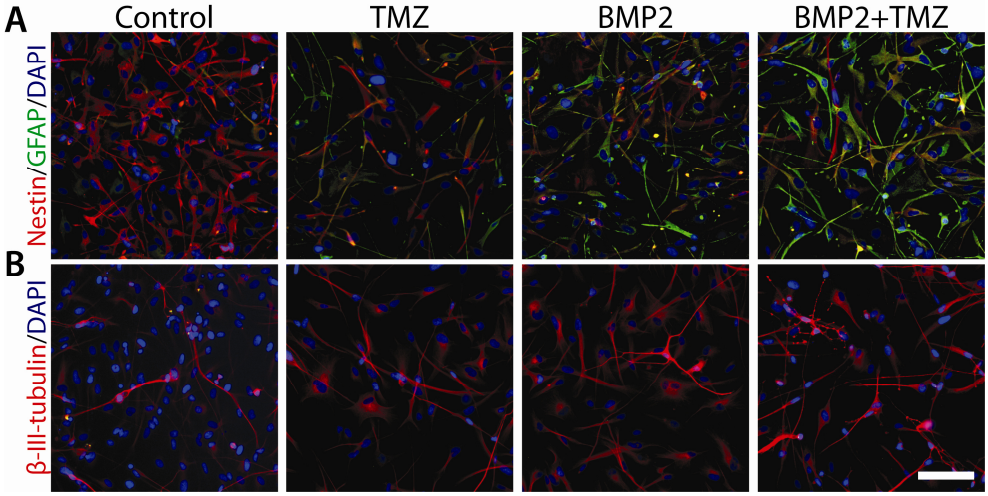




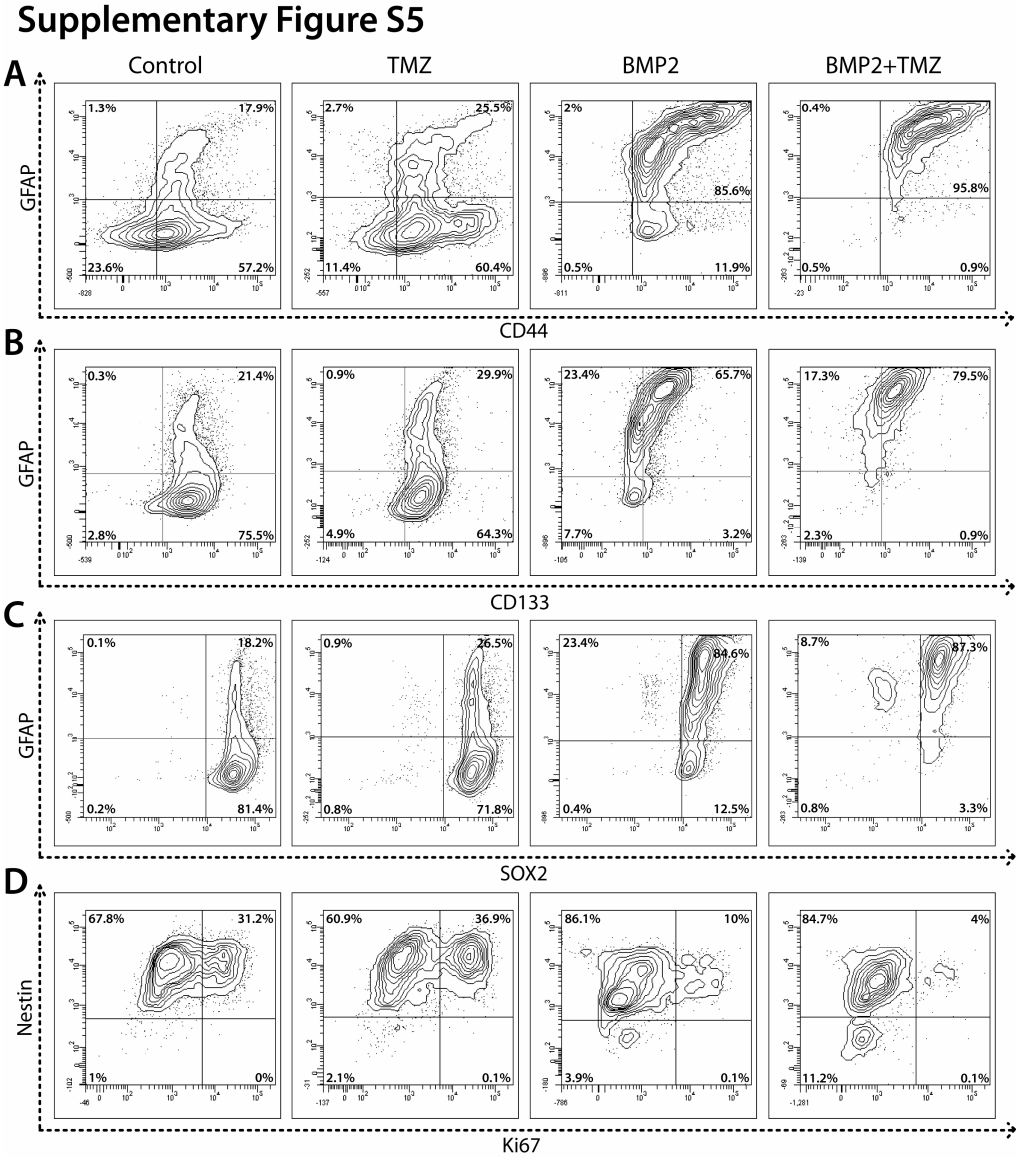
Supplementary Figure S3



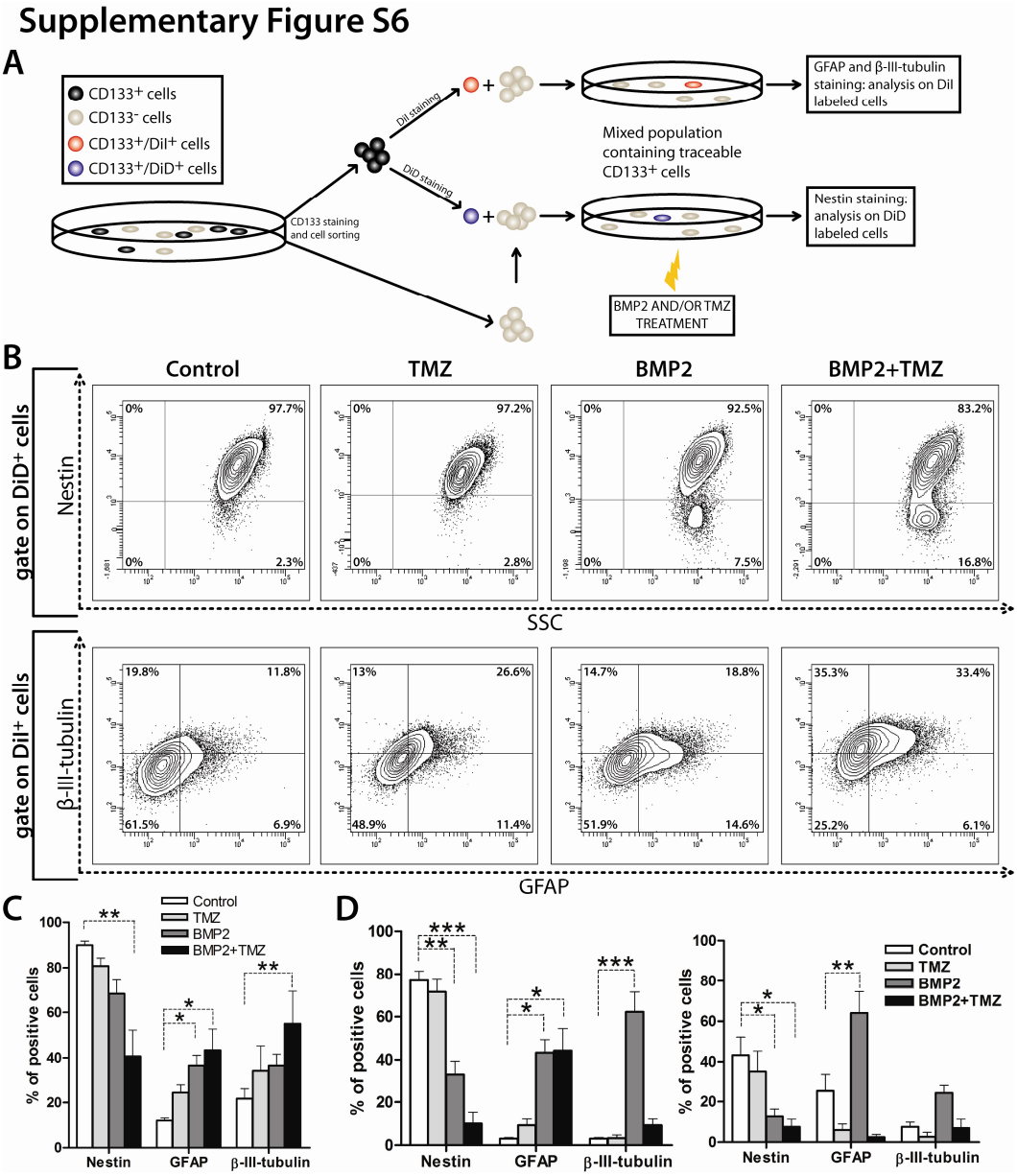
Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6



SUPPLEMENTARY TABLES

Supplementary Table S1. Brain Tumors used in the study

Code	Tumor Classification	Age	Gender
HuTuP51	Glioblastoma	57	Male
HuTuP52	Glioblastoma	53	Male
HuTuP53	Glioblastoma	70	Male
HuTuP55	Glioblastoma	58	Male
HuTuP56	Glioblastoma	46	Male
HuTuP60	Glioblastoma	58	Male
HuTuP61	Glioblastoma	70	Female
HuTuP64	Glioblastoma	61	Male
HuTuP67	Glioblastoma	49	Male
HuTuP83	Glioblastoma	55	Male

Brain tumours were acquired directly from surgery, two samples from each of the three concentric layers were obtained and part of the tissues was enzymatically dissociated. Single cells were cultured at 2%O2. Patient ages listed in years (y)

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Different phenotype of three distinct tumour layers-derived tissues. Representative immunofluorescence images (HuTuP120) of (A) Nestin(red)/GFAP (green), (C) β-III-tubulin(red), (E) HIF-1α(green) of tissue sections of biopsies directly derived from the three GBM layers and (B,D,F) bar graphs reporting relative quantification based on percentage of $DAPI^+$ cells. The graphs report mean of six tumors 6 ± SEM. Bar=40μm. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure S2. Phenotypic effects of pro-differentiating treatments on GBM cells derived from the core. (A) Representative immunofluorescence images of GBM cells (HuTuP64) derived from the dissociation of the core and treated for 5 days in absence of bFGF and EGF and in presence of: BMP2 alone (10ng/ml), B27 alone (2%), CNTF alone (10ng/ml), NT3 alone (10ng/ml), a combination of NT3 (10ng/ml), CNTF (10ng/ml) and B27 (2%), $LiCl_2$ alone (1mM) or FBS alone (1%). (B-D) Bar graphs reporting relative (B) $Nestin^+$, (C) $GFAP^+$ and (D) β-III-$tubulin^+$ cells quantification based on % of $DAPI^+$ cells. Mean ± S.E.M. comparing 3 different GBM, n=6 for each tumor. Bar = 40μm. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure S3. Effect of BMP2/TMZ treatment on proliferation of GBM cells derived from the core. (A) Representative immunocytochemical images of GBM cells derived from the dissociation of the core, treated with TMZ, BMP2 or the combination of both, and stained for Ki67 (red). (B) Bar graph showing percentage of cells in the different phases of the cell cycle measured with BrdU and 7-AAD staining. Bar=20μm *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure S4. Immunofluorescence of BMP2 and/or TMZ treated GBM cells. Representative immunofluorescence images of GBM cells (HuTuP56) derived from the dissociation of the core, stained for Nestin (red)/GFAP (green) (A) and for β-III-tubulin (red) (B) and treated

with TMZ, BMP2 or the combination of both. Arrowheads show the most neuronal differentiated cells in the field. Bar=40μm.

Supplementary Figure S5. Cytofluorimetric analysis of GBM differentiation after treatments. (A-D) Representative dot-plot analysis (HuTuP83) showing correlation of the surface expression of GFAP/CD44 (A), GFAP/CD133 (B), GFAP/Sox2 (C) and Nestin/Ki67 (D).

Supplementary Figure S6. Cytofluorimetric analysis of GBM stem-like cell differentiation after treatments. (A) GBM cells were stained for CD133, sorted and then labeled with membrane staining dyes (DiI or DiD; Invitrogen, Carlsbad, CA). Sorted and labeled cells were then used to generate mixed populations containing $CD133^+/Di^+$ cells and $CD133^-$ cells (1:10 ratio). Mixed populations were then treated with TMZ and/or BMP2 and stained for Nestin ($CD133^+/DiD^+$) or GFAP/β-III-tubulin ($CD133^+/DiI^+$). Analysis of cell phenotype and differentiation was measured on Di^+ or Di^- gated cells. (B) Representative dot-plot analysis (HuTuP13) showing correlation of the surface expression of DiD/Nestin (upper panels) or DiI/GFAP/β-III-tubulin (lower panels). (C) Bar graph showing differentiation of DiD^+ or DiI^+ $CD133^+$ GBM tracked cells by Nestin, GFAP and β-III-tubulin stainings after BMP2 and/or TMZ treatment. (D) Bar graphs reporting relative $Nestin^+$, $GFAP^+$ and β-III-$tubulin^+$ cell quantification in GBM cells derived from the core and sorted by FACS for the expression of CD133. The graphs report mean of 3 tumors ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$.